UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

December 21, 2005

MEMORANDUM

Subject: Efficacy Review for EPA Reg. No. 46781-6 *Cavicide;*
DP Barcode: D321712

From: Nancy Whyte, Acting Team Leader
Product Science Branch
Antimicrobials Division (7510C)

December 12, 2005

Michele E. Wingfield, Chief
Product Science Branch
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To: Marshall Swindell/Portia Jenkins
Team 33,Regulatory Management Branch I
Antimicrobials Division (7510C)

Applicant: Metrex Research Corporation
28210 Wick Road
Romulus, MI 48174

Formulation from the Label:

Active Ingredient(s)	% by wt
Diisobutylphenoxyethoxyethyl dimethyl benzyl ammonium chloride	0.28%
Isopropanol	17.2%*
Total	100.00%

*** At this concentration, isopropanol cannot be efficacious against microorganisms, and must be removed as an active ingredient from the ingredient claims statement**

I. BACKGROUND

The product, Cavicide (EPA Reg. No. 46781-6), is an EPA-approved disinfectant (bactericide, fungicide, tuberculocide, virucide) for use on hard, non-porous surfaces in commercial, institutional, food preparation, animal care, and hospital or medical environments. The applicant requested an amendment to the registration of this product to lower contact times for selected microorganisms and to add claims for effectiveness against a number of additional microorganisms (i.e., *Clostridium difficile*, *Staphylococcus aureus* VRSA, Influenza A2 virus). All studies were conducted at MicroBioTest, Inc., located at 105B Carpenter Drive in Sterling, VA 20164.

This data package contained a letter from the applicant to EPA (dated August 22, 2005), a letter from EPA to the applicant (dated February 8, 2005), ten studies (MRID Nos. 466309-01 through 466309-10), Statements of No Data Confidentiality Claims for all ten studies, the last accepted label (dated July 2, 2004), and the proposed label.

II USE DIRECTIONS

The product is designed for use in disinfecting hard, non-porous surfaces such as ambulance equipment and surfaces, animal cages, appliances, barber/salon instruments, bathroom fixtures, computers, counter tops, dental instruments and surfaces, diaper pails, eye glasses, floors, furniture (e.g., bassinets, chairs, cribs, high chairs, stools, tables), garbage cans, infant incubators, infant/child care equipment surfaces, laboratory equipment and surfaces, lamps, medical equipment and surfaces, physical therapy equipment, sinks, telephones, toilets, toys, veterinary equipment and surfaces, and walls. The product may be used on painted surfaces and surfaces made of plastic (i.e., polycarbonate, polypropylene, polyvinylchloride, polystyrene), non-porous vinyl, stainless steel, Plexiglas®, Formica®, or glass. Directions on the proposed label provided the following information regarding use of the product as a disinfectant: Spray product directly onto pre-cleaned surface, thoroughly wetting area to be disinfected. Allow surface to remain wet for 2 minutes at room temperature (69°F/20°C). Wipe surface using a towel, or allow to air dry. Product effectiveness against *Mycobacterium bovis* BCG, *Pseudomonas aeruginosa*, *Salmonella choleraesuis*, and *Staphylococcus aureus*, requires a 3-minute contact time.

The proposed label directions also included special instructions for disinfecting pre-cleaned, non-critical medical instruments: Thoroughly pre-clean instruments to remove excess organic debris. Rinse and dry. Clean and rinse lumens of hollow instruments. Using either a soaking tray or ultrasonic unit, immerse instruments into the product for 2 minutes. Remove and rinse instruments. Wipe dry prior to use. Product effectiveness against *Mycobacterium bovis* BCG, *Pseudomonas aeruginosa*, *Salmonella choleraesuis*, and *Staphylococcus aureus*, requires a 3-minute contact time.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

Disinfectants for Use as Tuberculocides (Using the AOAC Tuberculocidal Test Method, Tuberculocidal Activity of Disinfectants Test Method with modifications, or the AOAC Germicidal Spray Products as Disinfectants Method)

Disinfectants may bear additional label claims of effectiveness as tuberculocides when supported by appropriate tuberculocidal effectiveness data. Certain chemical classes (i.e., glutaraldehyde and quaternary ammonium compounds) are required to undergo validation testing in addition to basic testing. Products that are formulated with other chemical groups do not require validation testing. Products may be tested using one of four recommended

methods: the AOAC Tuberculocidal Test Method, Tuberculocidal Activity of Disinfectants Test Method with significant modification of the standard test conditions of contact time and/or temperature, Quantitative Tuberculocidal Activity Test Method, and AOAC Germicidal Spray Products as Disinfectants Method.

When using the existing or modified AOAC Tuberculocidal Activity Test Methods, or the AOAC Germicidal Spray Products as Disinfectants Method, 10 carriers for each of 2 samples, representing 2 different product lots, must be tested against *Mycobacterium bovis* BCG (a member of the *Mycobacterium tuberculosis* species complex). When using the existing or modified AOAC Tuberculocidal Activity Test Method, or the AOAC Germicidal Spray Products as Disinfectants Method, killing on all carriers/slides as demonstrated in Modified Proskauer-Beck Broth, and no growth in any of the inoculated tubes of 2 additional media (i.e., Middlebrook 7H9 Broth Difco B, Kirchner's Medium, and/or TB Broth Base) is required. Agency standards are presented in EPA DIS/TSS-6, Subdivision G Guidelines, and "EPA Data Call-in Notice for Tuberculocidal Claims," dated June 13, 1986.

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments (Additional Bacteria)

Effectiveness of disinfectants against specific bacteria other than those named in the AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Method, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method, must be determined by either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products as Disinfectants Method. Ten carriers must be tested against each specific microorganism with each of 2 product samples, representing 2 different product lots. To support products labeled as "disinfectants" for specific bacteria (other than those bacteria named in the above test methods), killing of the specific microorganism on all carriers is required. In addition, plate count data must be submitted for each microorganism to demonstrate that a concentration of at least 10^4 microorganisms survived the carrier-drying step. These Agency standards are presented in DIS/TSS-1.

Virucides

The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level. These Agency standards are presented in DIS/TSS-7.

Virucides – Novel Virus Protocol Standards

To ensure that a virus protocol has been adequately validated, data should be provided from at least 2 independent laboratories for each product tested (i.e., 2 batches per product per laboratory).

IV. COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. MRID 466309-01 "AOAC Tuberculocidal Activity of Disinfectants" for Cavicide, by Angela L. Hollingsworth. Study conducted at MicroBioTest, Inc. Study completion date – April 21, 2004. Laboratory Project Identification Number 198-294.

This study, under the direction of Study Director Felicia L. Sellers, was conducted against Mycobacterium bovis BCG. Three lots (Lot Nos. 3-1309, 3-2311, and 3-2324) of the product, Cavicide, were tested using the AOAC Confirmative in vitro Test for Determining Tuberculocidal Activity Method (modified) as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) porcelain penicylinder carriers per product lot were immersed in a 14-25 day old suspension of the test organism, at a ratio of 10 carriers per 15-20 mL of inoculum. The carriers were dried in an incubator for 30 minutes at 37±2°C. Each carrier was exposed to 10 mL of the product for 3 minutes at 20+2°C. After the contact period, individual carriers were neutralized in 10 mL of Modified Proskauer Beck Medium containing 7% Polysorbate 80 and 1% Lecithin. After at least 10 minutes in the neutralizer, each carrier was transferred to 20 mL of Modified Proskauer-Beck Medium. From each tube of neutralizer, 2 mL was subcultured to each of the two remaining recovery media – Kirchner's Medium and Middlebrook 7H9 Broth. Subcultures were incubated for 60 days at 37+2°C. Incubation was continued for an additional 30 days because no growth was observed. Post-incubation, the subcultures were observed for the presence or absence of growth. Controls included those for viability, neutralizer effectiveness, sterility, carrier counts, and confirmation of the challenge microorganism.
Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

2. MRID 466309-02 "Confirmatory AOAC Tuberculocidal Activity of Disinfectants" for Cavicide, by Angela L. Hollingsworth. Study conducted at MicroBioTest, Inc. Study completion date – July 23, 2004. Laboratory Project Identification Number 198-297.

This study, under the direction of Study Director Angela L. Hollingsworth, was conducted against Mycobacterium bovis BCG. Three lots (Lot Nos. 3-1309, 3-2311, and 3-2324) of the product, Cavicide, were tested using the AOAC Confirmative in vitro Test for Determining Tuberculocidal Activity Method (modified) as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. The product was received ready-to-use. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) porcelain penicylinder carriers per product lot were immersed in a 14-25 day old suspension of the test organism, at a ratio of 10 carriers per 15-20 mL of inoculum. The carriers were dried in an incubator for 30 minutes at 37±2°C. Each carrier was exposed to 10 mL of the product for 3 minutes at 20+2°C. After the contact period, individual carriers were neutralized in 10 mL of Modified Proskauer Beck Medium containing 7% Polysorbate 80 and 1% Lecithin. After at least 10 minutes in the neutralizer, each carrier was transferred to 20 mL of Modified Proskauer-Beck Medium. From each tube of neutralizer, 2 mL was subcultured to each of the two remaining recovery media – Kirchner's Medium and Middlebrook 7H9 Broth. Subcultures were incubated for 60 days at 37+2°C. Incubation was continued for an additional 30 days because no growth was observed. Post-incubation, the subcultures were again observed for the presence or

absence of growth. Controls included those for viability, neutralizer effectiveness, sterility, carrier counts, and confirmation of the challenge microorganism.
Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

3. MRID 466309-03 "AOAC Use-Dilution Test Using Clostridium difficile" for Cavicide, by Angela L. Hollingsworth. Study conducted at MicroBioTest, Inc. Study completion date – August 4, 2004. Laboratory Project Identification Number 198-311.

This study was conducted against Clostridium difficile (ATCC 9689). Two lots (Lot Nos. 4-1139 and 4-2111) of the product, Cavicide, were tested using the AOAC Use-Dilution Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. The product was received ready-to-use. Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed in a 48-54 hour old broth culture of the test organism, at a ratio of 20 carriers per 20 mL broth. The carriers were dried at 37+2°C for 20-40 minutes. Each carrier was exposed to 10 mL of the product for 2 minutes at 20°C. After exposure, each carrier was transferred to Reinforced Clostridial Medium containing 7% Polysorbate 80 and 1% Lecithin. All subcultures were incubated for 48+2 hours at 37+2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for sterility, neutralizer effectiveness, dried carrier counts, viability, bacteriostasis, and confirmation of the challenge microorganism.
Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

4. MRID 466309-04 "AOAC Use-Dilution Test Supplemental," Test Organism: Staphylococcus aureus with reduced susceptibility to vancomycin (ATCC 700699) for Cavicide, by Angela L. Hollingsworth. Study conducted at MicroBioTest, Inc. Study completion date – August 10, 2004. Laboratory Project Identification Number 198-312.

This study was conducted against Staphylococcus aureus with reduced susceptibility to vancomycin (ATCC 700699). Two lots (Lot Nos. 4-1139 and 4-2111) of the product, Cavicide, were tested using the AOAC Use-Dilution Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. The product was received ready-to-use. Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed in a 48-54 hour old broth culture of the test organism, at a ratio of 20 carriers per 20 mL broth. The carriers were dried at 37+2°C for 20-40 minutes. Each carrier was exposed to 10 mL of the product for 2 minutes at 20°C. After exposure, each carrier was transferred to Letheen Broth containing 7% Polysorbate and 1% Lecithin. All subcultures were incubated for 48+2 hours at 37+2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for sterility, neutralizer effectiveness, dried carrier counts, viability, bacteriostasis, antibiotic resistance, and confirmation of the challenge microorganism.
Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.
Note: Antibiotic resistance of Staphylococcus aureus with reduced susceptibility to vancomycin (ATCC 700699) was verified on a representative culture. Individual Mueller Hinton Agar was streaked with the microorganism in a crosshatch pattern. A vancomycin-impregnated disk was added to the center of the plate. The plate was incubated for 24+2 hours at 37+2□C. A

measured zone of inhibition of 13 mm was observed, confirming reduced *susceptibility* of the microorganism to vancomycin.

5. MRID 466309-05 "AOAC Use-Dilution Test Supplemental," Test Organisms: *Staphylococcus aureus* - MRSA (ATCC 33592) and *Enterococcus faecalis* VRE (ATCC 51299) for Cavicide, by Angela L. Hollingsworth. Study conducted at MicroBioTest, Inc. Study completion date – August 4, 2004. Laboratory Project Identification Number 198-299.

This study was conducted against *Staphylococcus aureus* - MRSA (ATCC 33592) and *Enterococcus faecalis* VRE (ATCC 51299). Two lots (Lot Nos. 4-1139 and 4-2111) of the product, Cavicide, were tested using the AOAC Use-Dilution Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. The product was received ready-to-use. Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed in a 48-54 hour old broth culture of the test organism, at a ratio of 20 carriers per 20 mL broth. The carriers were dried at 37+2°C for 20-40 minutes. Each carrier was exposed to 10 mL of the product for 2 minutes at 20°C. After exposure, each carrier was transferred to Letheen Broth containing 7% Polysorbate 80 and 1% Lecithin. All subcultures were incubated for 48+2 hours at 37+2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for sterility, neutralizer effectiveness, dried carrier counts, viability, bacteriostasis, antibiotic resistance, and confirmation of the challenge microorganisms.
Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.
Note: Antibiotic resistance of Staphylococcus aureus - MRSA and Enterococcus faecalis VRE was verified on representative cultures. Separate Mueller Hinton Agar plates were streaked with each microorganism in a crosshatch pattern. An appropriate antibiotic-impregnated disk was added to the center of each plate. The plates were incubated for 24+2 hours at 37+2□C. A measured zone of inhibition of 0 mm was observed using an oxacillin-impregnated disk for Staphylococcus aureus - MRSA and a measured zone of inhibition of 13 mm was observed using a vancomycin-impregnated disk for *Enterococcus faecalis* VRE. According to the laboratory report, these measured zones confirm antibiotic resistance.

6. MRID 466309-06 "Virucidal Effectiveness Test Using Bovine viral diarrhea virus (BVDV) (Surrogate for human Hepatitis C virus)" for Cavicide, by M. Khalid Ijaz. Study conducted at MicroBioTest, Inc. Study completion date – September 7, 2004. Amended report date – September 27, 2004. Laboratory Project Identification Number 198-303.

This study, under the direction of Study Director M. Khalid Ijaz, was conducted against the Bovine viral diarrhea virus (strain not specified; obtained from American BioResearch Laboratories) using MDBK cells (ATCC CCL-22; propagated in-house) as the host system. The study protocol followed MicroBioTest Protocol "Virucidal Effectiveness Test Using Bovine viral diarrhea virus (BVDV) (Surrogate for human Hepatitis C virus)," dated April 12, 2004 (copy provided). Two lots (Lot Nos. 4-1139 and 4-2111) of the product, Cavicide, were tested. The product was received ready-to-use. The stock virus culture contained at least a 5% organic soil load. Two glass carriers were tested for each product lot against the target virus. Films of virus were made by spreading 0.2 mL of stock virus on the bottoms of separate sterile glass Petri dishes. The virus films were dried for 30-60 minutes at room temperature. For each lot of product, 2.0 mL of the product was added to the virus films for 1 minute at 21°C. After the contact period, the virus-disinfectant mixture was neutralized with 2.0 mL of horse serum and the mixture was scraped from the surface of the dish with a cell scraper. Each sample was

loaded onto pre-spun Sephacryl columns and the eluate was removed. Ten-fold serial dilutions were prepared, using Minimum Essential Media Eagle's containing 5% horse serum. MDBK cells were inoculated in quadruplicate with an unspecified amount of selected dilutions and incubated at 37±2°C in 5±1% CO_2 for 3-7 days. Post-incubation, the plates were assayed by a direct immunofluorescence assay. The $\log_{10}$ reduction of infectious BVDV fluorescent focus-forming units was determined using the Most Probable Number (MPN) method. Controls included those for cell viability/sterility, virus stock titer, plate recovery, column titer, neutralizer effectiveness, cytotoxicity, cytotoxicity-related viral interference, and data consistency.
Note: The report was amended to correct the contact time.
Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

7. MRID 466309-07 "Confirmatory Virucidal Effectiveness Test Using Bovine Viral Diarrhea Virus (BVDV) (Surrogate for Human Hepatitis C virus)" for Cavicide, by Zheng Chen. Study conducted at MicroBioTest, Inc. Study completion date – October 13, 2004. Laboratory Project Identification Number 198-304.

This study, under the direction of Study Director Zheng Chen, was conducted against the Bovine viral diarrhea virus (strain not specified; obtained from American BioResearch Laboratories) using MDBK cells (ATCC CCL-22; propagated in-house) as the host system. The study protocol followed MicroBioTest Protocol "Confirmatory Virucidal Effectiveness Test Using Bovine viral diarrhea (BVDV) (Surrogate for human Hepatitis C virus)," dated April 12, 2004 (copy provided). One lot (Lot No. 4-1139) of the product, Cavicide, was tested. The product was received ready-to-use. The stock virus culture contained at least a 5% organic soil load. Two glass carriers were tested for the single product lot against the target virus. Films of virus were made by spreading 0.2 mL of stock virus on the bottoms of separate sterile glass Petri dishes. The virus films were dried for 30-60 minutes at room temperature. For the single product lot, 2.0 mL of the product was added to the virus films for 1 minute at 22°C. After the contact period, the virus-disinfectant mixture was neutralized with 2.0 mL of horse serum and the mixture was scraped from the surface of the dish with a cell scraper. Each sample was loaded onto pre-spun Sephacryl columns and the eluate was removed. Ten-fold serial dilutions were prepared, using Minimum Essential Medium Eagle's containing 5% horse serum. MDBK cells were inoculated in quadruplicate with an unspecified amount of selected dilutions and incubated at 37±2°C in 5±1% CO_2 for 3-5 days. Post-incubation, the plates were assayed by a direct immunofluorescence assay. The $\log_{10}$ reduction of infectious BVDV fluorescent focus-forming units was determined using the Most Probable Number (MPN) method. Controls included those for cell viability/sterility, virus stock titer, plate recovery, column titer, neutralizer effectiveness, cytotoxicity, cytotoxicity-related viral interference, and data consistency.
Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

8. MRID 466309-08 "Virucidal Efficacy Test, Human Influenza Virus" for Cavicide, by M. Khalid Ijaz. Study conducted at MicroBioTest, Inc. Study completion date – July 30, 2004. Laboratory Project Identification Number 198-308.

This study was conducted against Influenza A2 virus, Japan/305/57 (H2N2) strain (obtained from Charles River Laboratories; propagated in-house), using embryonated chicken eggs (obtained from B&E Eggs) as the host system. Two lots (Lot Nos. 4-1139 and 4-2111) of the product, Cavicide, were tested according to MicroBioTest Protocol "Virucidal Efficacy Test, Human influenza virus," dated April 12, 2004 (copy provided). The product was received ready-to-use. The stock virus culture contained at least a 5% organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile

glass Petri dishes. The virus films were dried for 30-60 minutes at room temperature. For each lot of product, separate dried virus films were treated with 2.0 mL of the product for 30 seconds at 22°C. After exposure, 2.0 mL of fetal bovine serum was added to each Petri dish. The plates were then scraped with a cell scraper to re-suspend the contents. Each sample was loaded onto pre-spun Sephacryl columns and the eluate was removed. The virus-disinfectant mixture was diluted serially in Earle's Balanced Salt Solution. Fertilized, embryonated chicken eggs (age not specified) were inoculated in quadruplicate via the allantoic route with 0.2 mL of selected dilutions. The eggs were incubated at 37±2□C for 5-7 days. The embryos were candled 1-day post-inoculation to determine the viability of the embryos. Eggs containing dead embryos were discarded. Following the incubation period, the eggs were candled and then kept at 2±2□C overnight. Afterwards, the allantoic fluid was harvested and stored at 2±2□C. The samples were assayed for the presence of replicating virus using an hemagglutination assay. Controls included those for host viability/sterility, virus stock titer, plate recovery, column titer, toxicity, toxicity-related viral interference, and neutralizer effectiveness. The 50% embryo lethal dose/ embryo infectious dose per mL (ELD/EID_{50}/mL) was determined using the method of Reed and Muench.

9. MRID 466309-09 "Initial Virucidal Effectiveness Test Using Duck Hepatitis B Virus (DHBV)" for Cavicide, by M. Khalid Ijaz. Study conducted at MicroBioTest, Inc. Study completion date – September 27, 2004. Laboratory Project Identification Number 198-301.

This study, under the direction of Study Director M. Khalid Ijaz, was conducted against the Duck Hepatitis B virus (obtained from HepadnaVirus Testing; propagated in-house) using primary duck hepatocytes (prepared from ducklings obtained from Metzer Farms) as the host system. The study protocol followed MicroBioTest Protocol "Initial Virucidal Effectiveness Test Using Duck Hepatitis B Virus," dated April 12, 2004 (copy provided). Two lots (Lot Nos. 4-1139 and 4-2111) of the product, Cavicide, were tested. The product was received ready-to-use. The viral stock contained at least a 5% organic soil load. Two glass carriers were tested for each product lot against the target virus. Films of virus were made by spreading 0.2 mL of stock virus on the bottoms of separate sterile glass Petri dishes. The virus films were dried for 30-60 minutes at room temperature. For each lot of product, 2.0 mL of the product was added to the dried virus films for 2 minutes at 20±2°C. After the contact period, the virus-disinfectant mixture was neutralized with 2.0 mL of fetal bovine serum and the mixture was scraped from the surface of the dish with a cell scraper. Each sample was loaded onto pre-spun Sephacryl columns and the eluate was removed. Ten-fold serial dilutions were prepared, using Liebovitz-15 complete tissue culture medium (CCM). Primary duck hepatocytes were inoculated in quadruplicate with an unspecified amount of each dilution and incubated at 37±2°C in 5±1% CO_2 for 20-30 hours for viral adsorption. Post-adsorption, the media were aspirated and the cells were washed and re-fed with CCM. The host cells then were incubated at 37±2°C in 5±1% CO_2 for an additional 7-14 days. The plates were assayed by immunofluorescence assay. The log_{10} reduction of infectious DHBV fluorescent focus-forming units was determined using the Most Probable Number (MPN) method. Controls included those for cell viability/sterility, virus stock titer, plate recovery, column titer, neutralizer effectiveness, cytotoxicity, cytotoxicity-related viral interference, and data consistency.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: The Quality Assurance Unit Statement on page 4 of the laboratory report references Project Number 198-303, not Project Number 198-301.

10. MRID 466309-10 "Confirmatory Virucidal Effectiveness Test Using Duck Hepatitis B Virus" for Cavicide, by Zheng Chen. Study conducted at MicroBioTest, Inc. Study completion date – October 19, 2004. Laboratory Project Identification Number 198-302.

This study, under the direction of Study Director Zheng Chen, was conducted against the Duck Hepatitis B virus (obtained from HepadnaVirus Testing; propagated in-house), using primary duck hepatocytes (prepared from ducklings obtained from Metzer Farms) as the host system. The study protocol followed MicroBioTest Protocol "Confirmatory Virucidal Effectiveness Test Using Duck Hepatitis B Virus," dated April 12, 2004 (copy provided). One lot (Lot No. 4-1139) of the product, Cavicide, was tested. The product was received ready-to-use. The viral stock contained at least a 5% organic soil load. Two glass carriers were tested for the single product lot against the target virus. Films of virus were made by spreading 0.2 mL of stock virus on the bottoms of separate sterile glass Petri dishes. The virus films were dried for 30-60 minutes at room temperature. For the single product lot, 2.0 mL of the product was added to the dried virus films for 2 minutes at 21°C. After the contact period, the virus-disinfectant mixture was neutralized with 2.0 mL of fetal bovine serum and the mixture was scraped from the surface of the dish with a cell scraper. Each sample was loaded onto pre-spun Sephacryl columns and spun to obtain the eluate. Ten-fold serial dilutions were prepared, using Liebovitz-15 complete tissue culture medium (CCM). Primary duck hepatocytes were inoculated in quadruplicate with an unspecified amount of each dilution and incubated at 37±2°C in 5±1% CO_2 for 20-30 hours for viral adsorption. Post-adsorption, the media were aspirated and the cells were washed and re-fed with CCM. The host cells then were incubated at 37±2°C in 5±1% CO_2 for an additional 7-14 days. The plates were assayed by an immunofluorescence assay. The $\log_{10}$ reduction of infectious DHBV fluorescent focus-forming units was determined using the Most Probable Number (MPN) method. Controls included cell viability/sterility, virus stock titer, plate recovery, column titer, neutralizer effectiveness, cytotoxicity, cytotoxicity-related viral interference, and data consistency.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

See Following Pages for Results Tables

V RESULTS

MRID Number	Organism	Lot No.	No. of Carriers Showing Growth in Recovery Medium*			
			MPBM+	MPBM	7H9	KM
466309-01	*Mycobacterium bovis* BCG Carrier counts: 2.3×10^4 CFU/carrier	3-1309	0/10	0/10	0/10	0/10
		3-2311	0/10	0/10	0/10	0/10
		3-2324	0/10	0/10	0/10	0/10
466309-02	*Mycobacterium bovis* BCG Carrier counts: 1.3×10^5 CFU/carrier	3-1309	0/10	0/10	0/10	0/10
		3-2311	0/10	0/10	0/10	0/10
		3-2324	0/10	0/10	0/10	0/10

*MPBM = Modified Proskauer Beck Medium; MPBM+ = Modified Proskauer Beck Medium containing 7% Polysorbate 80 and 1% Lecithin; 7H9 = Middlebrook 7H9 Broth; KM = Kirchner's Medium

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested		Dried Carrier Count (CFU/ carrier)
		Lot No. 4-1139	Lot No. 4-2111	
466309-03	**Clostridium difficile**	0/10	0/10	1.3×10^4
466309-04	*Staphylococcus aureus* with reduced susceptibility to vancomycin	0/10	0/10	9.5×10^4
466309-05	*Staphylococcus aureus* - MRSA	0/10	0/10	8.0×10^4
466309-05	*Enterococcus faecalis* VRE	0/10	0/10	8.0×10^4

MRID Number	Organism	Results			Plate Recovery Control
			Lot No. 4-1139	Lot No. 4-2111	

MRID Number	Organism	Results			Plate Recovery Control
			Lot No. 4-1139	Lot No. 4-2111	
466309-06	Bovine viral diarrhea virus	10^{-2} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	6.0589 log_{10}MPN/mL
		log_{10}MPN/mL	0.0	0.0	
466309-07	Bovine viral diarrhea virus	10^{-2} to 10^{-7} dilutions	Complete inactivation	---	6.0589 log_{10}MPN/mL
		log_{10}MPN/mL	0.0	---	
466309-08	Influenza A2 virus	10^{-2} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$\geq 10^{6.23}$ ELD/EID_{50}/mL
		ELD/EID_{50}/mL	$\leq 10^{1.50}$	$\leq 10^{1.50}$	
466309-09	Duck Hepatitis B virus	10^{-2} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	5.0589 log_{10}MPN/mL
		log_{10}MPN/mL	0.0	0.0	
466309-10	Duck Hepatitis B virus	10^{-2} to 10^{-7} dilutions	Complete inactivation	---	5.2194 log_{10}MPN/mL
		log_{10}MPN/mL	0.0	---	

VI CONCLUSIONS

1. The submitted efficacy data (MRID Nos. 466309-01 and 466309-02) support the use of the product, Cavicide, as a disinfectant with tuberculocidal activity against *Mycobacterium bovis* BCG on hard, non-porous surfaces in the presence of a 5% organic soil load (heat-inactivated horse serum) for a contact time of 3 minutes. No growth was observed in any of the recovery media for any of the 10 carriers per 3 product lots tested. Neutralizer effectiveness testing showed positive growth of the microorganism. Viability controls were positive for growth. Sterility controls did not show growth. Required validation testing was performed at the same laboratory but under different study directors.

2. The submitted efficacy data support the use of the product, Cavicide, as a disinfectant against the following bacteria in the presence of a 5% organic soil load (heat-inactivated horse serum) on hard, non-porous surfaces for a contact time of 2 minutes:

Clostridium difficile	MRID No. 466309-03
Staphylococcus aureus with reduced susceptibility to vancomycin	MRID No. 466309-04
Enterococcus faecalis VRE	MRID No. 466309-05
Staphylococcus aureus - MRSA	MRID No. 466309-05

Acceptable killing was observed in the subcultures of carriers tested against the required number of product lots (i.e., 2 product lots). Dried carrier counts were at least 10^4. Neutralizer effectiveness testing showed positive growth of the microorganisms. Viability controls were positive for growth. Sterility controls did not show growth.

3. The submitted efficacy data support the use of the product, Cavicide, as a disinfectant with virucidal activity against the following microorganisms on hard, non-porous surfaces in the presence of a 5% organic soil load for the contact time listed:

Duck Hepatitis B virus (a surrogate for the Human Hepatitis B virus)	2 minutes	MRID Nos. 466309-09 and -10
Bovine viral diarrhea virus (a surrogate for the Hepatitis C virus)	1 minute	MRID Nos. 466309-06 and -07

Complete inactivation (no growth) was observed in all dilutions tested. Based on the plate recovery controls, it appears that recoverable virus titers of at least 10^4 were achieved. Initial and confirmatory studies were performed at the same laboratory but under different study directors. Confirmatory studies used one lot of product, not the standard two.

4. The submitted efficacy data (MRID No. 466309-08) support the use of the product, Cavicide, as a disinfectant with virucidal activity against Influenza A2 virus on hard, non-porous surfaces in the presence of a 5% organic soil load for a contact time of 30 seconds. Based on the plate recovery control, a recoverable virus titer of at least 10^4 was achieved. Complete inactivation (no growth) was observed in all dilutions tested.

VII RECOMMENDATIONS

The applicant revised the last accepted label (dated July 2, 2004) in part to lower contact times. This data package included studies supporting lower contact times for use of the product as a disinfectant against *Mycobacterium bovis* BCG, *Staphylococcus aureus* – MRSA, *Enterococcus faecalis* VRE, Hepatitis B virus, and Hepatitis C virus. Efficacy data previously submitted to the Agency supports lower contact times for other microorganisms (i.e., Herpes simplex virus type 1, Herpes simplex virus type 2, Human immunodeficiency virus type 1). The applicant failed to provide studies supporting lower contact times for all microorganisms listed on the last accepted label (e.g., *Trichophyton mentagrophytes*, Human coronavirus). Appendix I of this efficacy report presents a tabular summary of label revisions and whether (or not) the applicant provided efficacy data to support lower contact times.

A. *Recommendations Regarding Efficacy Claims*

1. The proposed label claims that the product, Cavicide, is an effective disinfectant on pre-cleaned, hard, non-porous surfaces against the following microorganisms for a contact time of 3 minutes:

   Mycobacterium bovis BCG
   Pseudomonas aeruginosa
   Salmonella choleraesuis
   Staphylococcus aureus

   Data provided by the applicant (either in this data package or as part of previous submissions to the Agency) support these claims.

2. The proposed label claims that the product, Cavicide, is an effective disinfectant on pre-cleaned, hard, non-porous surfaces against the following microorganisms for a contact time of 2 minutes:

   Clostridium difficile
   Enterococcus faecalis VRE
   Staphylococcus aureus - MRSA
   Hepatitis B virus
   Hepatitis C virus
   Herpes simplex virus type 1
   Herpes simplex virus type 1
   Human immunodeficiency virus type 1
   Influenza A2 virus

   Data provided by the applicant (either in this data package or as part of previous submissions to the Agency) support these claims.

3. The proposed label claims that the product, Cavicide, is an effective disinfectant on applicant has not provided data to support this claim. Efficacy data were provided for the challenge microorganism identified as *Staphylococcus aureus*, with reduced susceptibility to vancomycin. As noted in the laboratory report assigned MRID No. 466309-04, only reduced susceptibility of the microorganism to vancomycin was verified in an antibiotic resistance study. The applicant needs to revise the proposed label so that all references to *Staphylococcus aureus* VRSA are changed to *Staphylococcus aureus* with reduced susceptibility to vancomycin.

4. The proposed label claims that the product, Cavicide, is an effective disinfectant on pre-cleaned, hard, non-porous surfaces against the following microorganisms for a contact time of 2 minutes:

   Trichophyton mentagrophytes
   Human coronavirus

   The applicant has not provided data to support these claims. Efficacy data previously submitted to EPA show product effectiveness at contact times greater than 2 minutes. See Appendix I of this efficacy report.

B. Miscellaneous Recommendations

1. In the letter from EPA to the applicant (dated February 8, 2005), the applicant was instructed to "delete[s] any efficacy directions for soaking." The proposed label (see page 5 of the proposed label) still includes directions for soaking and immersion in the "For Disinfecting Precleaned Non-Critical Medical Devices, Instruments and Implements . . ." section.

2. The proposed label includes the following new uses: [Kitchen and Bath Solution][for Hard, Non-porous Surfaces] and [Cleans, Deodorizes, and Kills Common Household Germs]. These new uses are seemingly inconsistent with language on the last accepted label and the proposed label, which state that the product is "For Professional Use Only." The applicant needs to revise the proposed label so that it is clear whether the product may be used in household environments.

3. The label must add the following statement to the claim for the effectiveness of the product against *Clostridium difficile* that the product has been tested only against the vegetative cells only, not against the spores.

4. The label claim for the product's effectiveness as a disinfectant agains Human Coronavirus does not imply that the product is effective against the causative agent of Severe Acute Respiratory Syndrome (SARS).

APPENDIX I

Challenge Organism	**Contact Time**		**Comments**
	Last Accepted Label (dated June 15, 2004)	**Proposed Label (dated August 22, 2005)**	
Mycobacterium bovis *BCG*	5 minutes	3 minutes	Lower contact time of 3 minutes was demonstrated. [See MRID Nos. 466309-01 and 02.]
Staphylococcus aureus	3 minutes	3 minutes	Contact time on proposed label unchanged.
Pseudomonas aeruginosa	3 minutes	3 minutes	Contact time on proposed label unchanged.
Salmonella choleraesuis	3 minutes	3 minutes	Contact time on proposed label unchanged.
Clostridium difficile	Not listed	2 minutes	Contact time of 2 minutes was demonstrated. [See MRID No. 466309-03.]
Trichophyton mentagrophytes	3 minutes	2 minutes	Lower contact time of 2 minutes was not demonstrated. As per EPA's Wallace Powell, DER D292554 (11/10/03) indicates that the laboratory study (MRID not

Challenge Organism	Contact Time		Comments
	Last Accepted Label (dated June 15, 2004)	Proposed Label (dated August 22, 2005)	
			specified) supported a 3-minute contact time.
Staphylococcus aureus MRSA	3 minutes	2 minutes	Lower contact time of 2 minutes was demonstrated. [See MRID No. 466309-05.]
Enterococcus faecalis VRE	3 minutes	2 minutes	Lower contact time of 2 minutes was demonstrated. [See MRID No. 466309-05.]
Staphylococcus aureus VRSA	Not listed	2 minutes	Contact time of 2 minutes was demonstrated. [See MRID No. 466309-04.] Efficacy data were provided for *Staphylococcus aureus* with reduced susceptibility to vancomycin, not for *Staphylococcus aureus* VRSA.
Hepatitis B virus	3 minutes	2 minutes	Lower contact time of 2 minutes was demonstrated. [See MRID Nos. 466309-09 and -10.]
Hepatitis C virus	3 minutes	2 minutes	Contact time of 1 minute was demonstrated. [See MRID Nos. 466309-06 and -07.]
Herpes simplex virus type 1	3 minutes	2 minutes	Contact time of 30 seconds was demonstrated. [See MRID Nos. 440277-12 and perhaps MRID Nos. 440277-14 and -15. As per EPA's Wallace Powell, DER D237859 (4/17/00).]
Herpes simplex virus type 2	3 minutes	2 minutes	Contact time of 30 seconds was demonstrated. [See MRID No. 440277-13 and perhaps MRID Nos. 440277-14 and -15. As per EPA's Wallace Powell, DER D237859 (4/17/00).]
Human immunodeficiency virus type 1	3 minutes	2 minutes	Lower contact time of 2 minutes was demonstrated. [See MRID No. 420358-02 and perhaps MRID No. 424677-01. As per EPA's Wallace Powell, DER D237859 (4/17/00).]
Human coronavirus	Not listed. 3 minutes (as per letter from EPA to applicant (dated February	2 minutes	Lower contact time of 2 minutes was not demonstrated. [See MRID No. 461438-01. As per EPA's Wallace Powell, DER D302065 (8/27/04 original review and 2/8/05

Challenge Organism	Contact Time		Comments
	Last Accepted Label (dated June 15, 2004)	Proposed Label (dated August 22, 2005)	
	8, 2005)		add-on).]
Influenza A2 virus	Not listed	2 minutes	Contact time of 30 seconds was demonstrated. [See MRID No. 466309-08.]